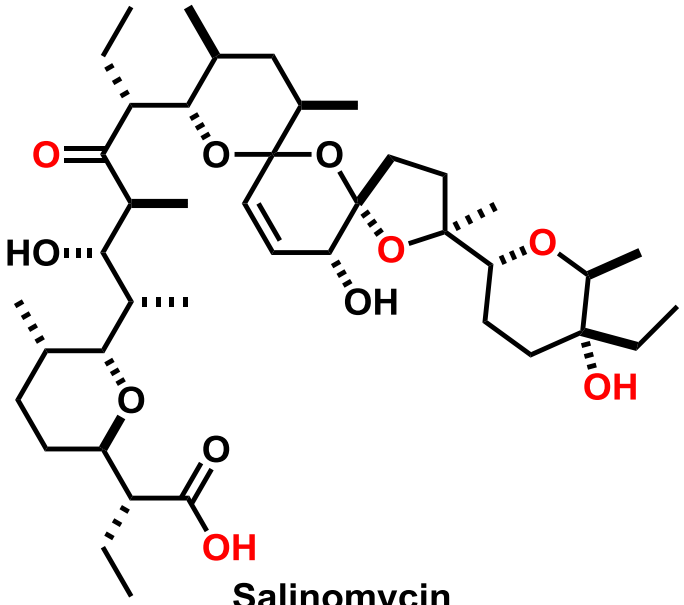
Salinomycin Kills Cancer Stem Cells by Sequestering Iron in Lysosomes



Salinomycin

20170527 Atsushi Hayata

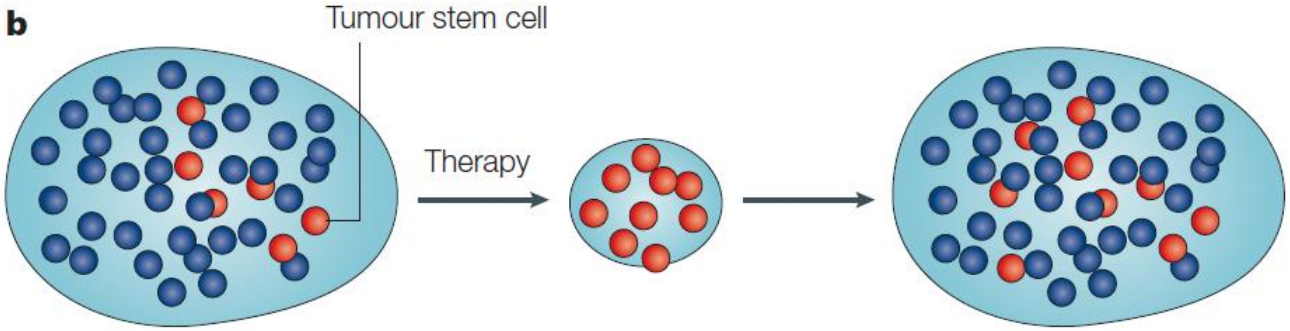
Contents

(1) Gupta, P. B.; Onder, T. T.; Jiang, G.; Tao, K.; Kuperwasser, C.; Weinberg, R. A.; Lander, E. S. *Cell* **2009** *138*, 645. (2) Mai, T. T.; Hamai, A.; Hienzsch, A.; Caneque, T.; Muller, S.; Wicinski, J.; Cabaud, O.; Leroy, C.; David, A.; Acevedo, V.; Ryo, A.; Ginestier, C.; Birnbaum, D.; Charage-Jauffret, E.; Codogno, P.; Mehrpour, M. Rodriguez, R. *Nat. Chem.* **2017** DOI: 10.1038/NCHEM.2778

1-1. Cancer Stem Cells (CSCs)

Cancer Stem Cells (CSCs) are subpopulations of cells within tumors that drive tumor growth and recurrence. 1)
CSC representation is operationally measured based on the ability to seed tumors at limiting dilutions in vivo.
CSCs are resistant to many current cancer treatments.

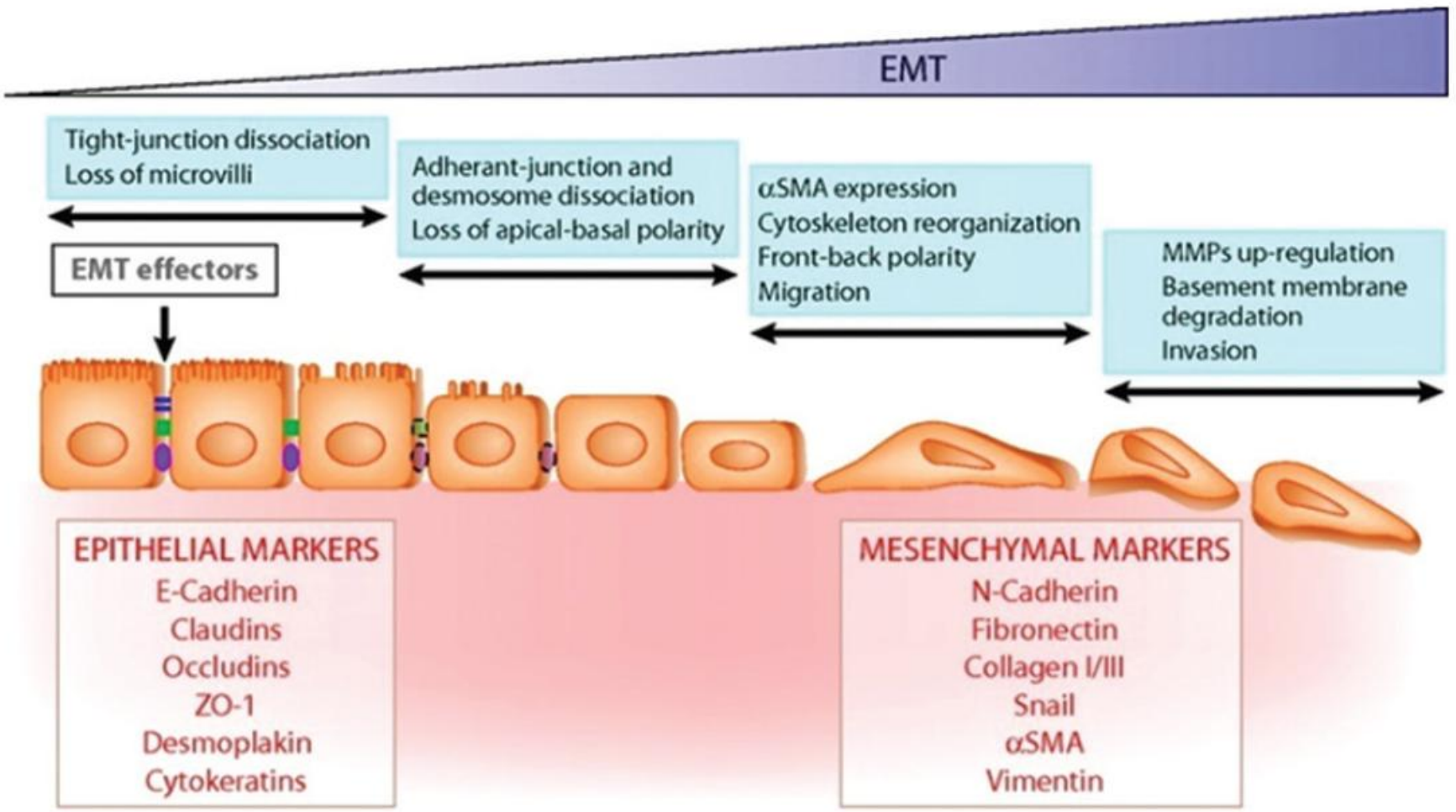
Model of tumor drug resistance 2)



(1) Gupta, P. B.; Onder, T. T.; Jiang, G.; Tao, K.; Kuperwasser, C.; Weinberg, R. A.; Lander, E. S. *Cell* **2009** *138*, 645. (2) Dean, M.; Fojo, T.; Bates, S. *Nature Reviews Cancer* **2005**, *5*, 275.

1-2. Epithelial-mesenchymal transition (EMT)

1)

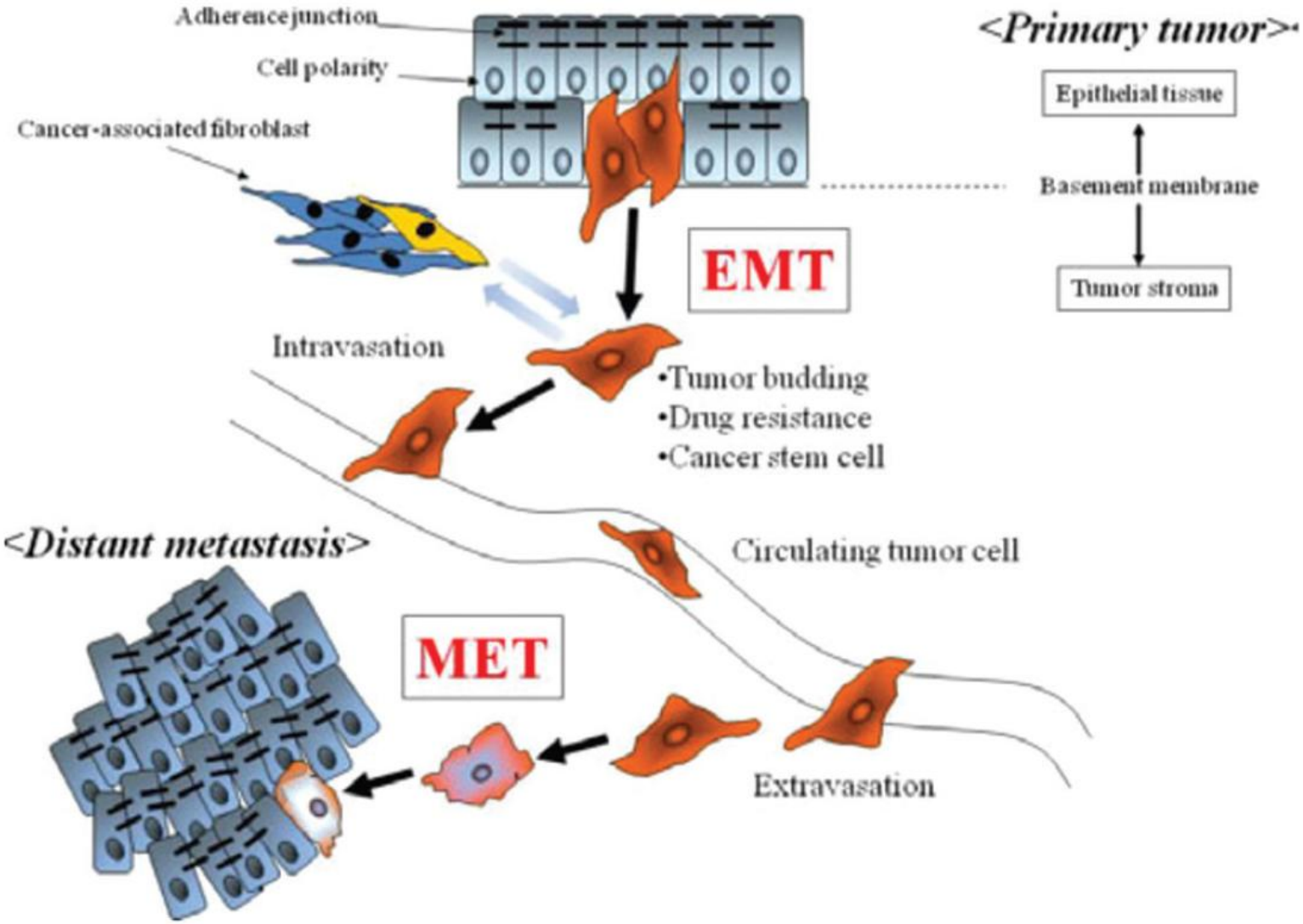


- **Embryogenesis**
- **Wound healing**
- **Cancer metastasis**

(1) Angadi, P. V.; Kale, A. D. *Indian Journal of Health Sciences* **2015**, *8*, 77.

1-2. EMT in cancer metastasis

1)



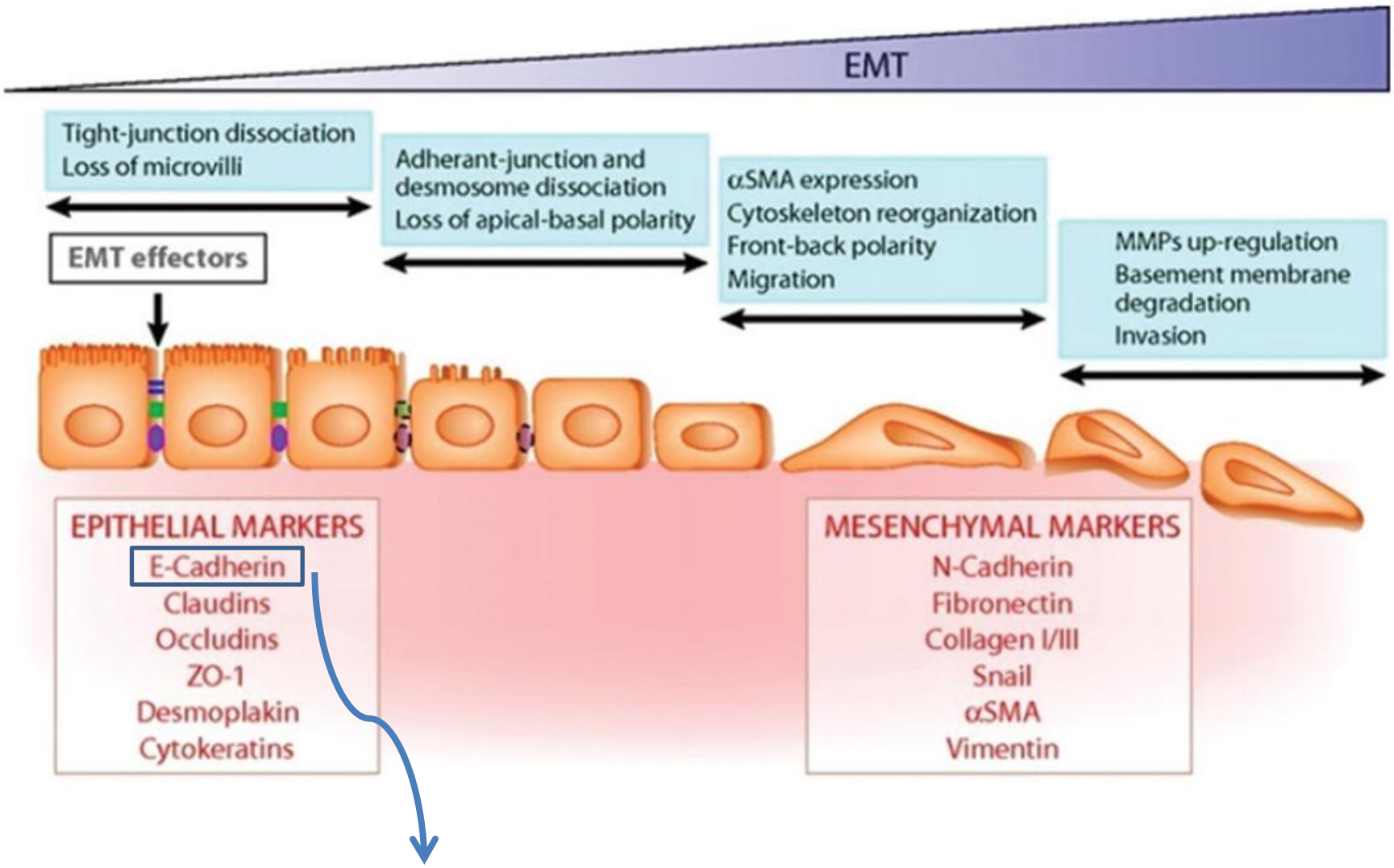
ability to seed tumors at limiting dilutions

-> cancer stem cell?

(1) Angadi, P. V.; Kale, A. D. *Indian Journal of Health Sciences* **2015**, *8*, 77.

1-2. Artificial induction of EMT

1)



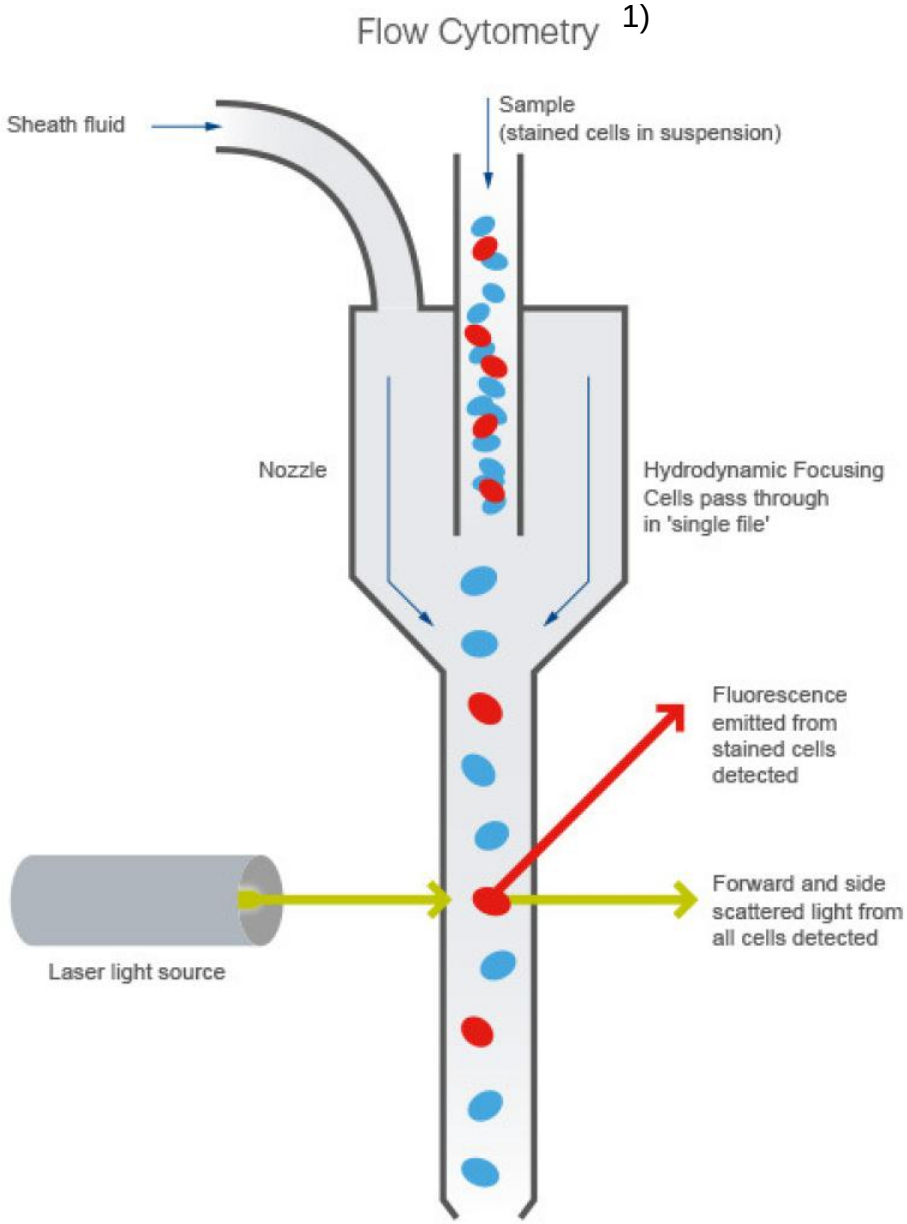
Down-regulation of E-cadherin by short hairpin RNA triggered an EMT.

(1) Angadi, P. V.; Kale, A. D. *Indian Journal of Health Sciences* **2015**, *8*, 77.

1-3. $CD44^{+}/CD24^{-}$ phenotype

In human breast cancers, CSCs are enriched in cells with $CD44^{+}/CD24^{-}$ phenotype.

CD24, CD44: cell adhesion molecule

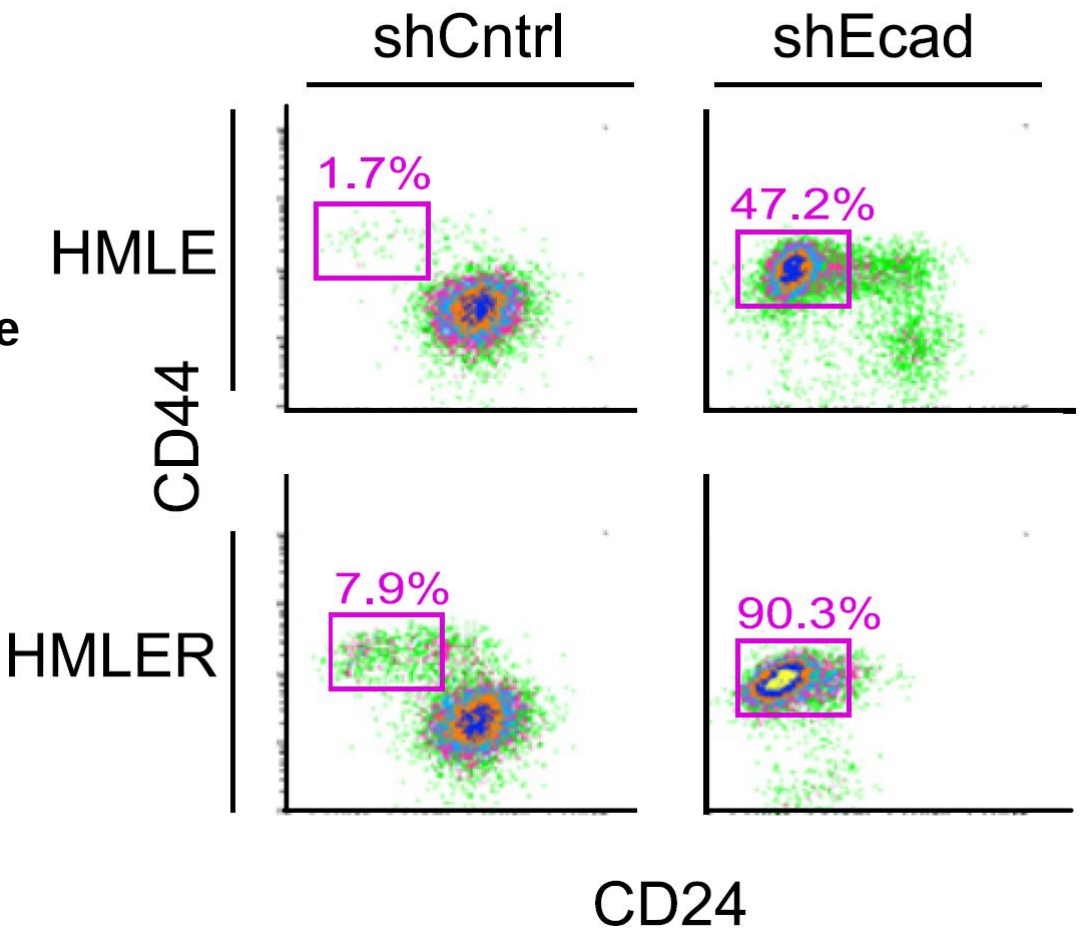


(1) Fillmore, C. M.; Kuperwasser, C. *Breast Cancer Research 10*, R25.

1-3. CD44^{+}/CD24^{-} phenotype

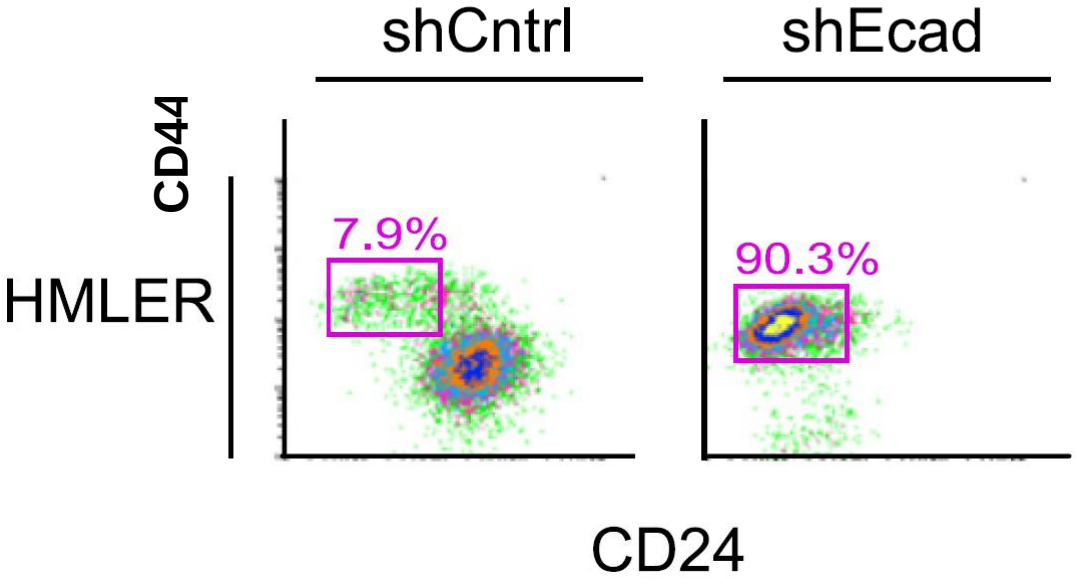
In human breast cancers, CSCs are enriched in cells with CD44^{+}/CD24^{-} phenotype.

CD24, CD44: cell adhesion molecule



HMEC: human mammary epithelial cell (normal cell)
HMLE: HMEC expressing LT and *hTERT*. (normal cell lines)
HMLER: HMEC expressing LT, *hTERT* and H-*ras*V12. (cancer cell lines)

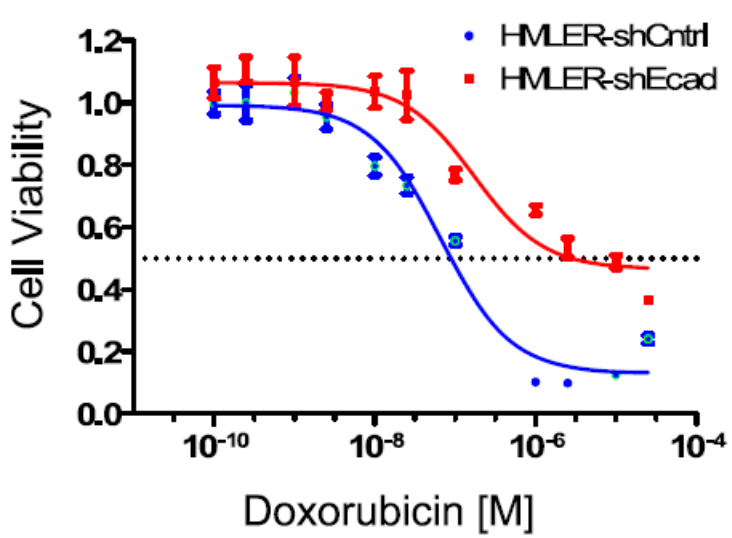
1-4. Model of human breast CSCs

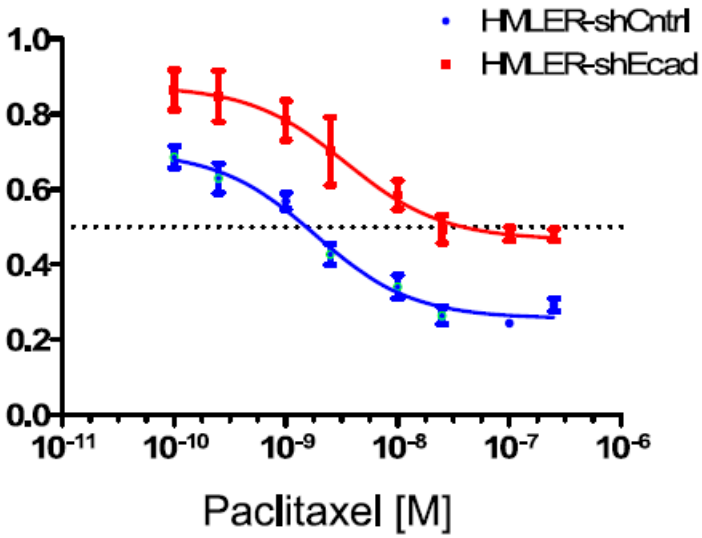


ability to seed tumors at limiting dilutions

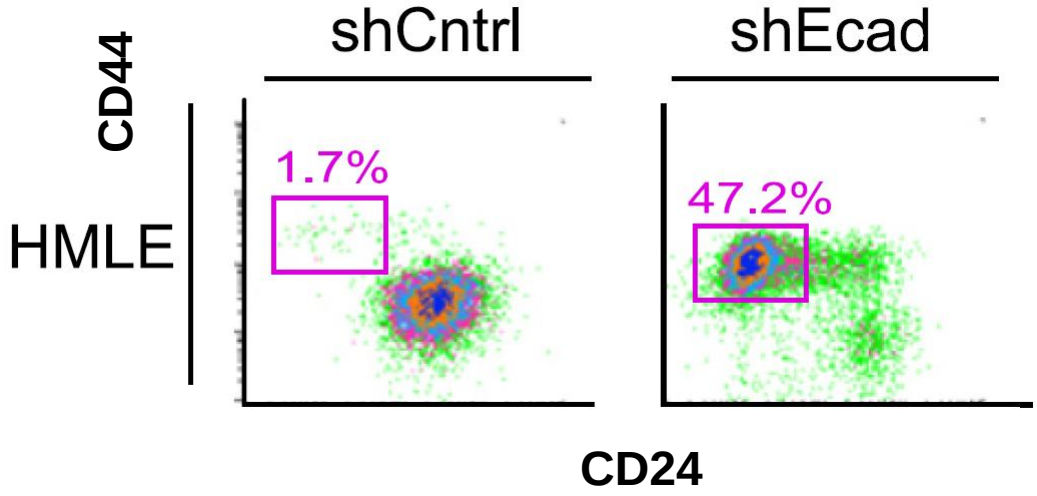
Tumor Seeding		
Cells injected	HMLER shCntrl	HMLER shEcad
$1x10^6$	8/10	10/10
$1x10^5$	3/4	4/4
$1x10^4$	1/4	4/4
$1x10^3$	0/4	4/4

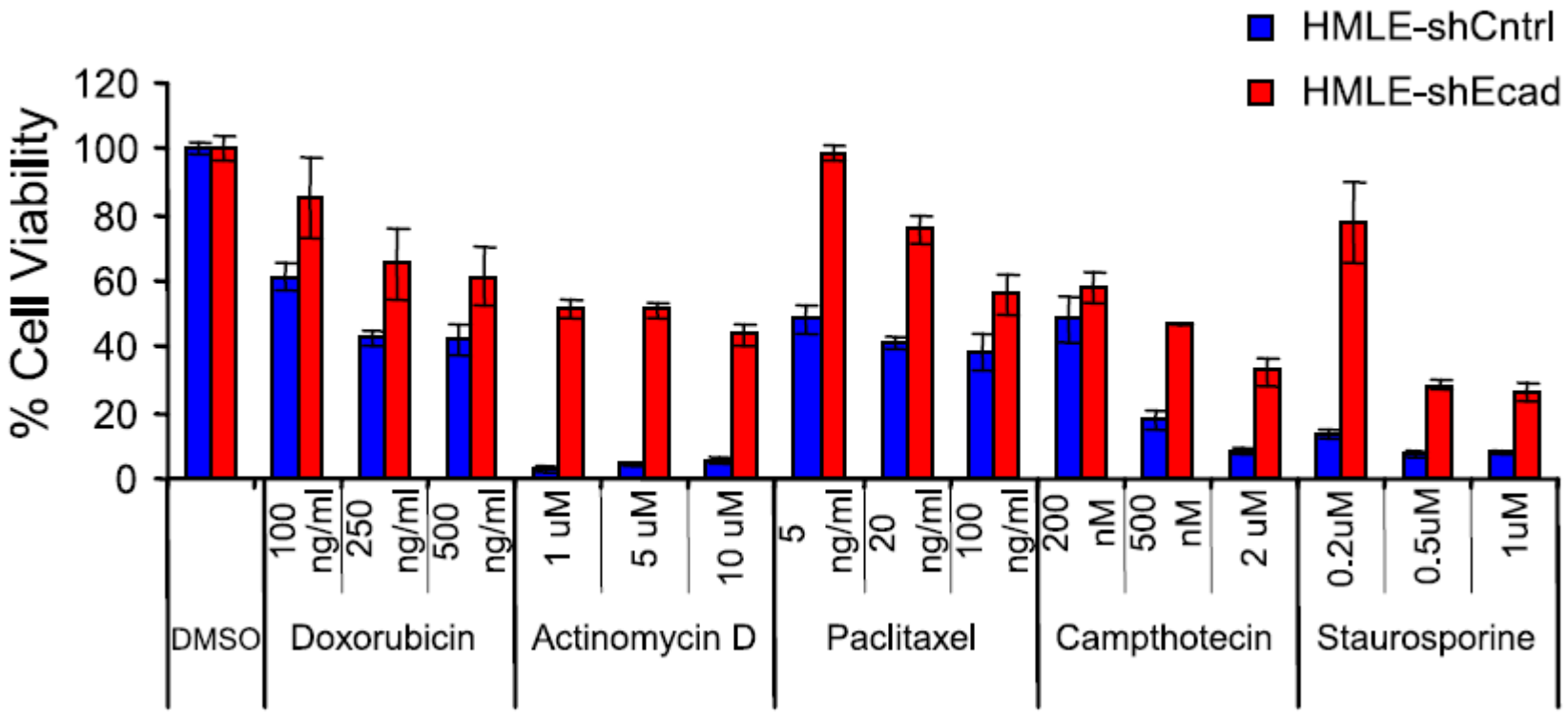
resistant to chemotherapeutic drugs





1-4. Model of human breast CSCs

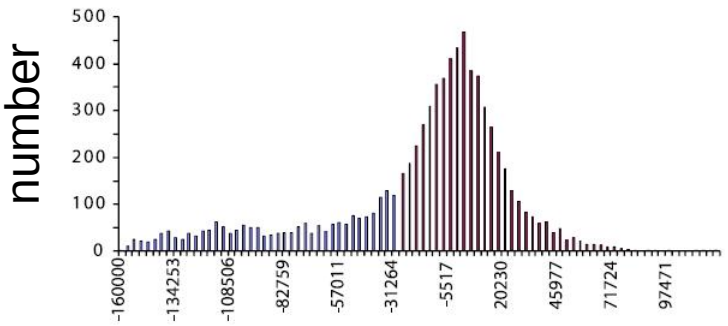


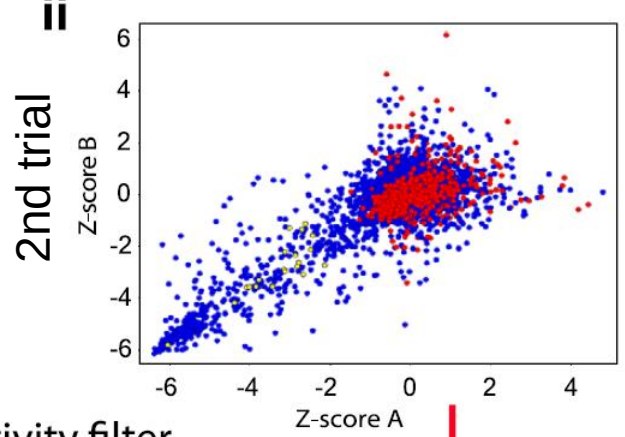


1-5. High-Throughput Screening (HTS)

HMLE^{shCntrl}
stem-depleted

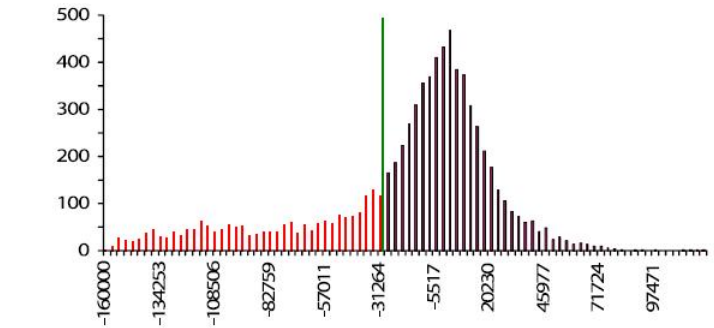
HMLE^{shEcad}
stem-enriched

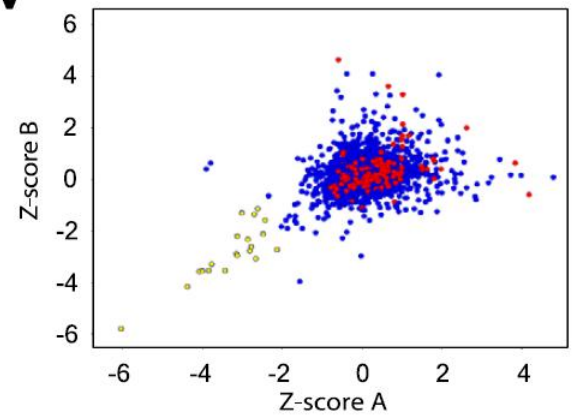




Apply selectivity filter

1st trial





~16000 compounds

HTS

32 compounds

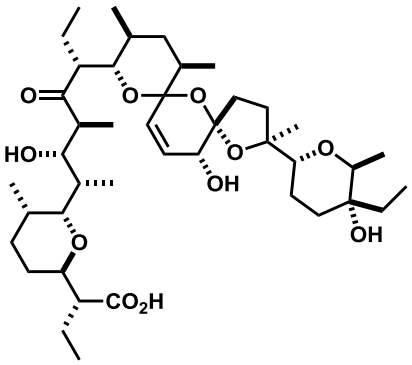
availability

8 compounds

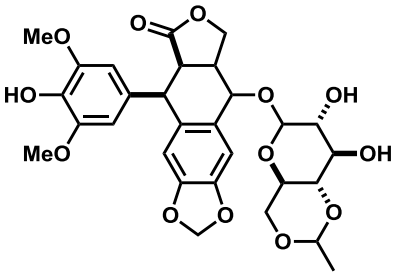
retest

4 compounds

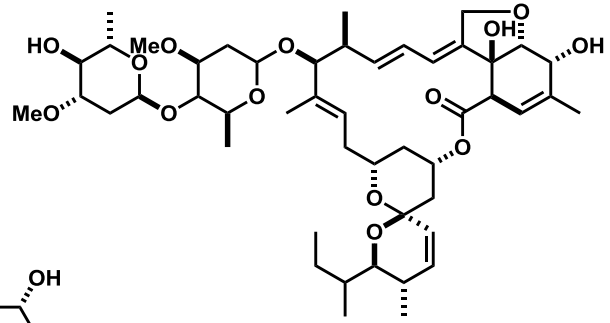
1-5. Hit 4 Compounds



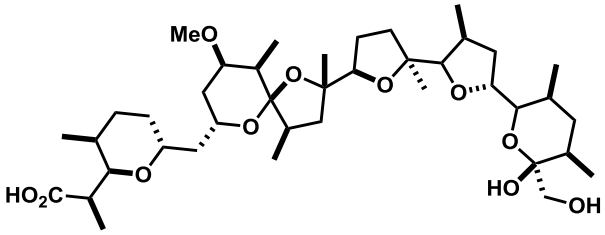
Salinomycin



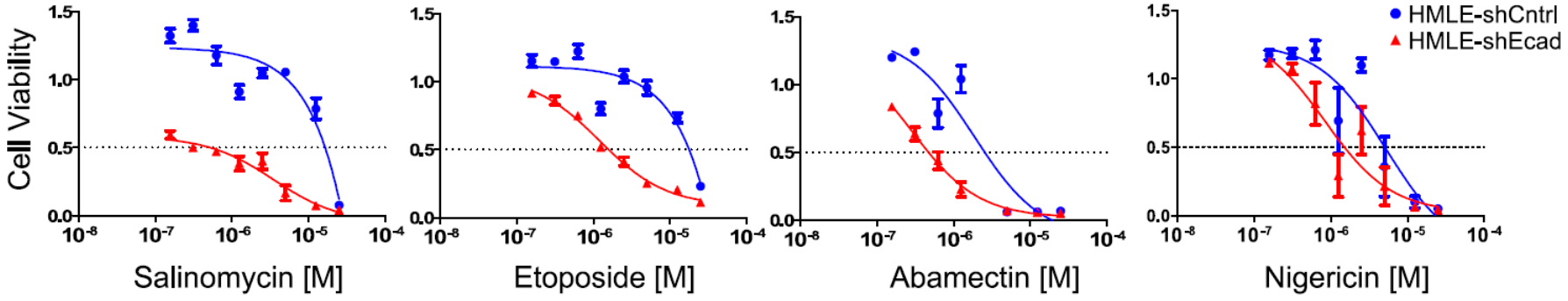
Etoposide

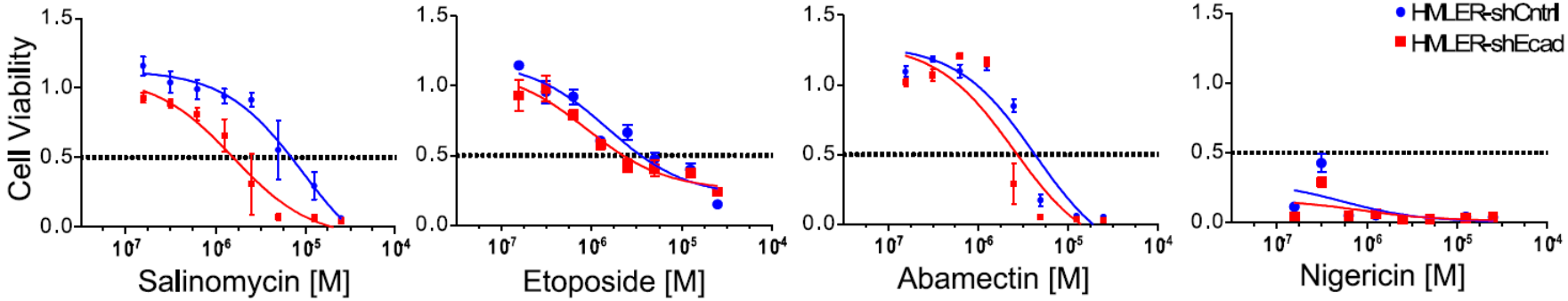


Abamectin

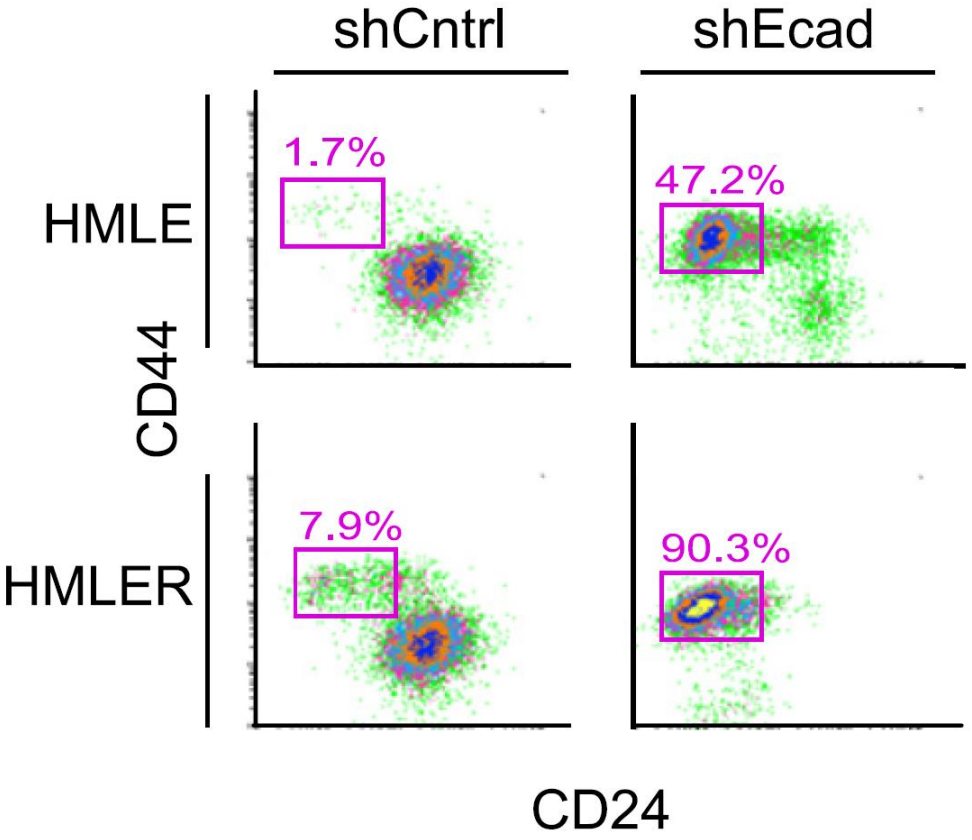


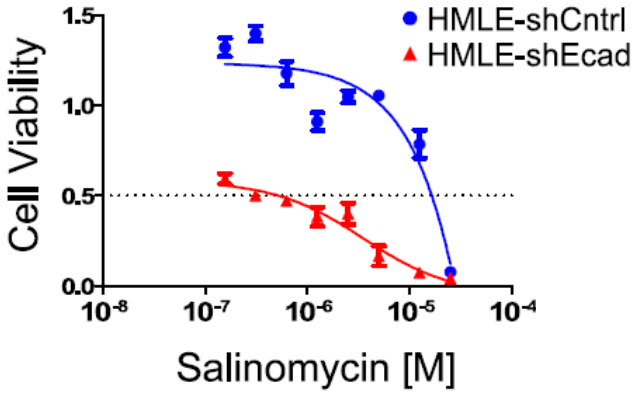
Nigericin

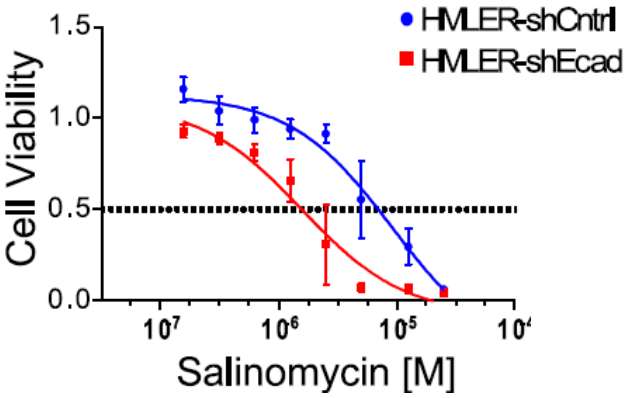




1-6. Short Summary







- **Establish CSC model cells with $CD44^{+}/CD24^{-}$ phenotype.**
- **Salinomycin selectively kills CSC model cells in vitro.**

- **Salinomycin was also effective than paclitaxel in vivo.**
- **Mechanisms iunduces CSC-specific toxicity remains unclear.**

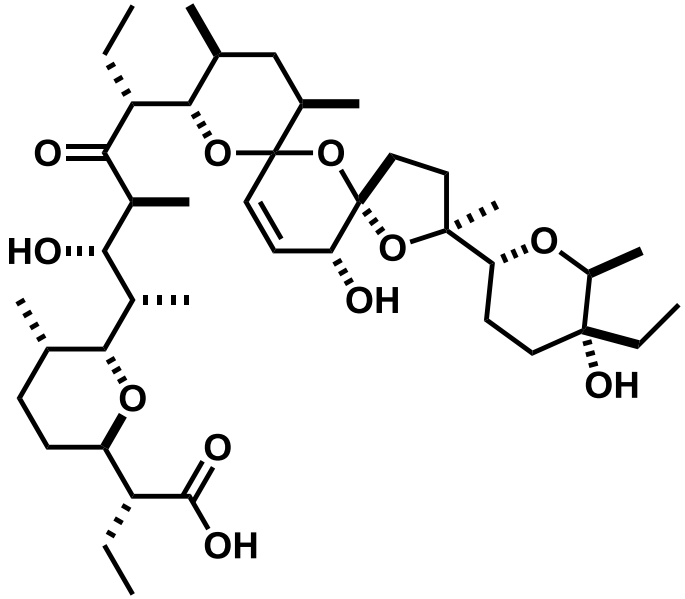
Contents

1. Identification of Selective Inhibitors of Cancer Stem Cells by High-Throughput Screening [1)]

2. Salinomycin Kills Cancer Stem Cells by Sequestering Iron in Lysosomes (main paper) [2)]

(1) Gupta, P. B.; Onder, T. T.; Jiang, G.; Tao, K.; Kuperwasser, C.; Weinberg, R. A.; Lander, E. S. *Cell* **2009** *138*, 645. (2) Mai, T. T.; Hamai, A.; Hienzsch, A.; Caneque, T.; Muller, S.; Wicinski, J.; Cabaud, O.; Leroy, C.; David, A.; Acevedo, V.; Ryo, A.; Ginestier, C.; Birnbaum, D.; Charage-Jauffret, E.; Codogno, P.; Mehrpour, M. Rodriguez, R. *Nat. Chem.* **2017** DOI: 10.1038/NCHEM.2778

2-1. Salinomycin



Salinomycin

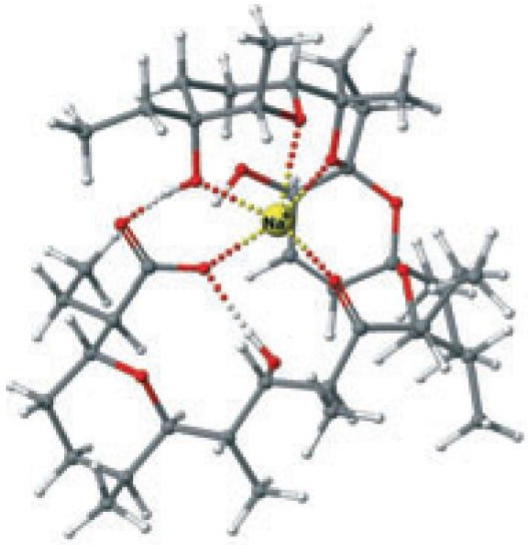
Isolation: from *Streptomyces albus*, ATCC 21838. [1)]

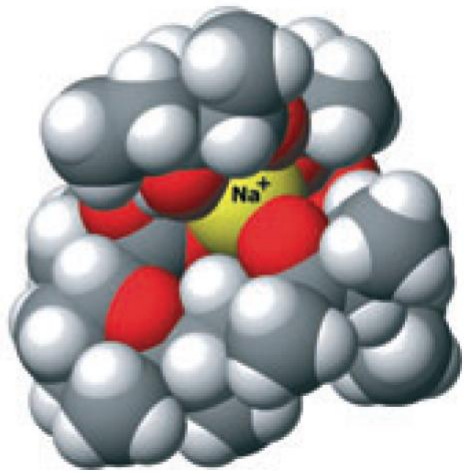
Biological activity: Antibiotics [1)]

Total synthesis: (a) Kishi, Y. (b) Yonemitsu, O. (c) Kocienski, P. J.[2)]

Widely used as coccidiostat (additive in feedstuff)

Structure of salinomycin-Na^+ complex. [3)]
(monovalent cation ionophore)

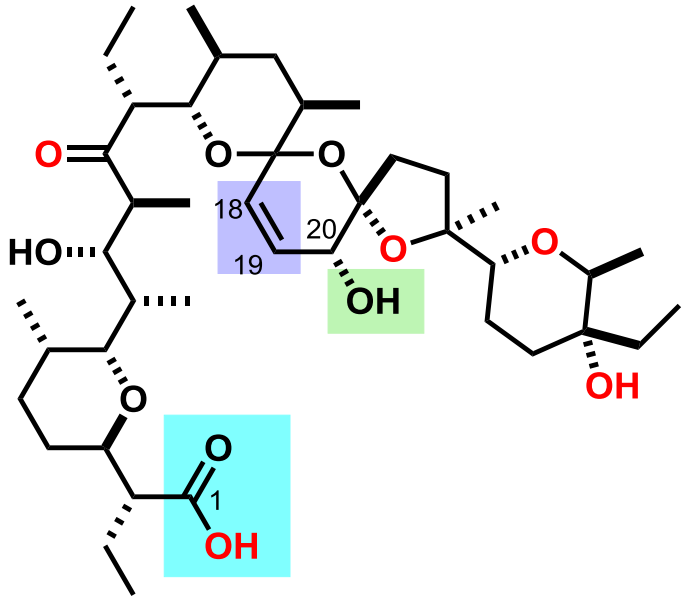




(1) Miyazaki, Y.; Shibuya, M.; Sugawara, H.; Kawaguchi, O.; Hirose, C.; Nagatu, J.; Esumi, S. *J. Antibio* **1974**, *28*, 814. (2) (a) Kishi, Y.; Hatakeyama, S.; Lewis, M. D. *Frontiers of Chemistry, ed. K. J. Laidler*, Oxford, **1982**. (b) Horita, K.; Oikawa, Y.; Yonemitsu, O. *Chem. Pharm. Bull.* **1989**, *37*, 1698. (c) Kocienski, P. J.; Brown, R. C. D.; Pommier, A.; Procter, M.; Schmidt, B. *J. Chem. Soc. Perkin Trans* **1998**, *1*, 9. (3) (a) Paulus, E. F.; Kurz, M.; Matter, H.; Vertesy, L. *J. Am. Chem. Soc.* **1998**, *120*, 8209. (b) Huczynski, A. *Chem. Biol. Drug Des.* **2012**, *79*, 235.

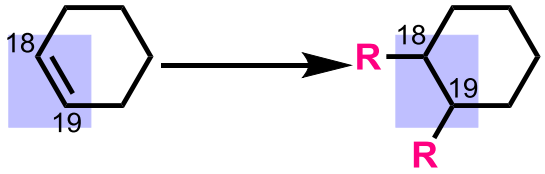
2-2. Salinomycin Derivatives

working hypothesis		
cytotoxicity	against normal or cancer cells	against cancer stem cells
mechanism	ionophore (no selectivity)	other unknown mechanism (selectivity)



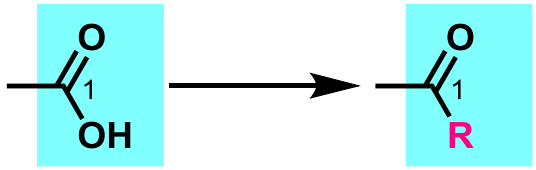
Salinomycin

C18-19 derivatives (2 compounds)



C18-19 derivatives show the weak cytotoxicity against CSC model cells.[1)]

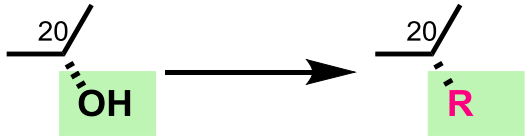
C1 derivatives (3 compounds)



ester, amide

C1 derivatives show the weak cytotoxicity against cancer cells.[2)]

C20 derivatives (14 compounds)



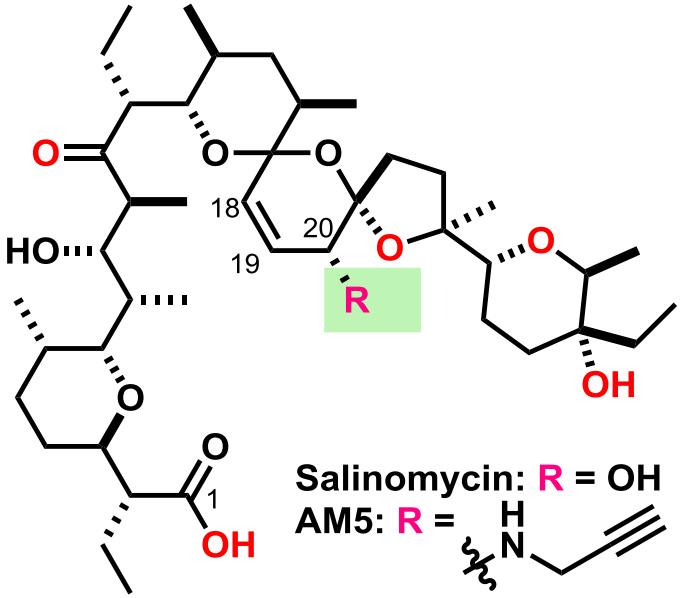
amine, ketone, oxyme

C20 derivatives show the strong cytotoxicity against cancer cells.[3)]

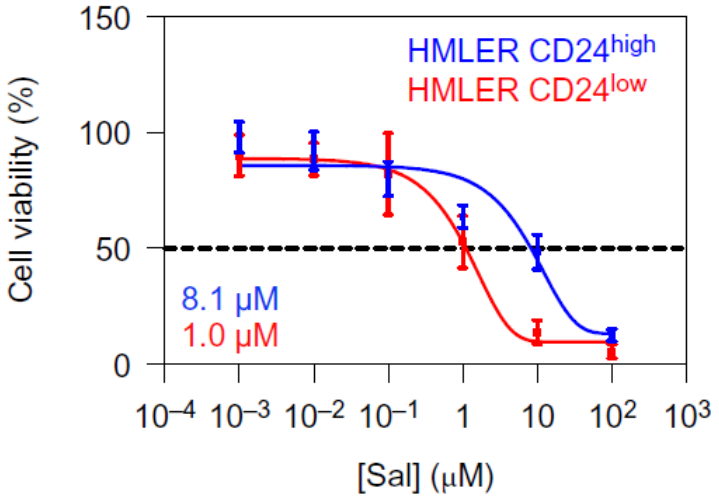
C1 and C20 derivatives (5 compounds)

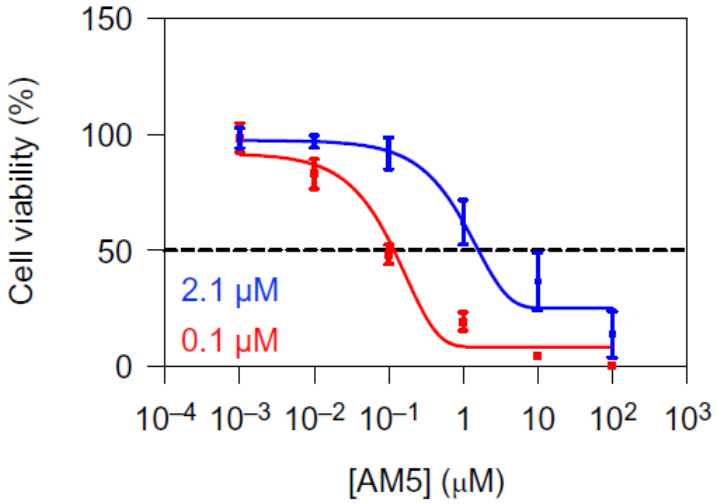
(1) Huang, X.; Borgstorm, B.; Mansson, L.; Persson, L.; Oredsson, S.; Hegardt, C.; Strand, D. *ACS Chem. Biol.* **2014**, *9*, 1587. (2) Huczynski, A.; Janczak, J.; Antoszczak, M.; Wietrzyk, J.; Maj, E. ; Brzezinski, B. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 7146. (3) Borgstorm, B.; Huang, X.; Posta, M.; Hegardt, C.; Oredsson, S.; Strand, D. *Chem. Commun.* **2013**, *49*, 9944.

2-3. Promising Derivative AM5

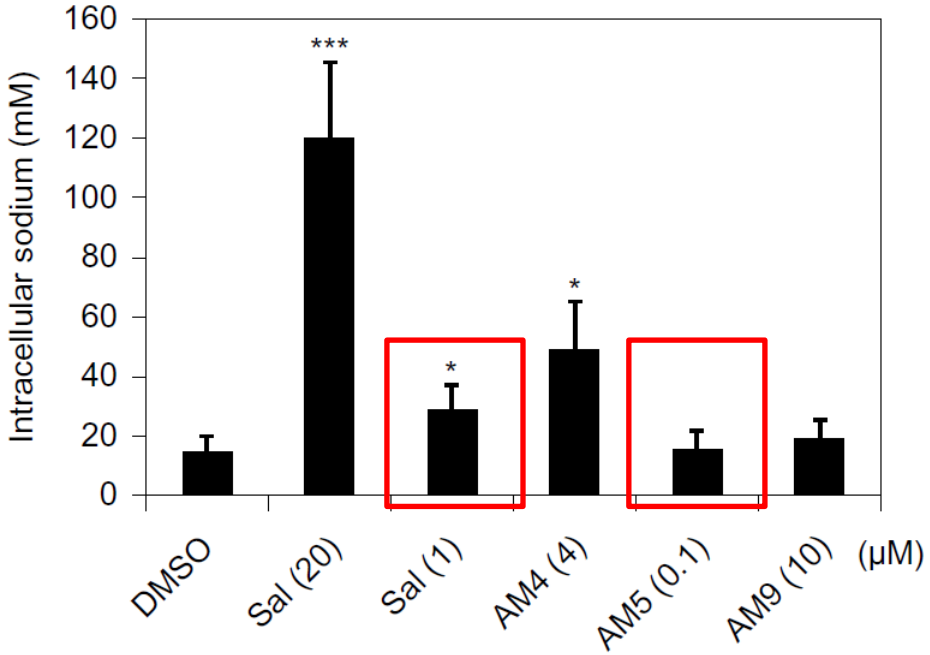


Cytotoxicity against cancer cells and model CSCs.

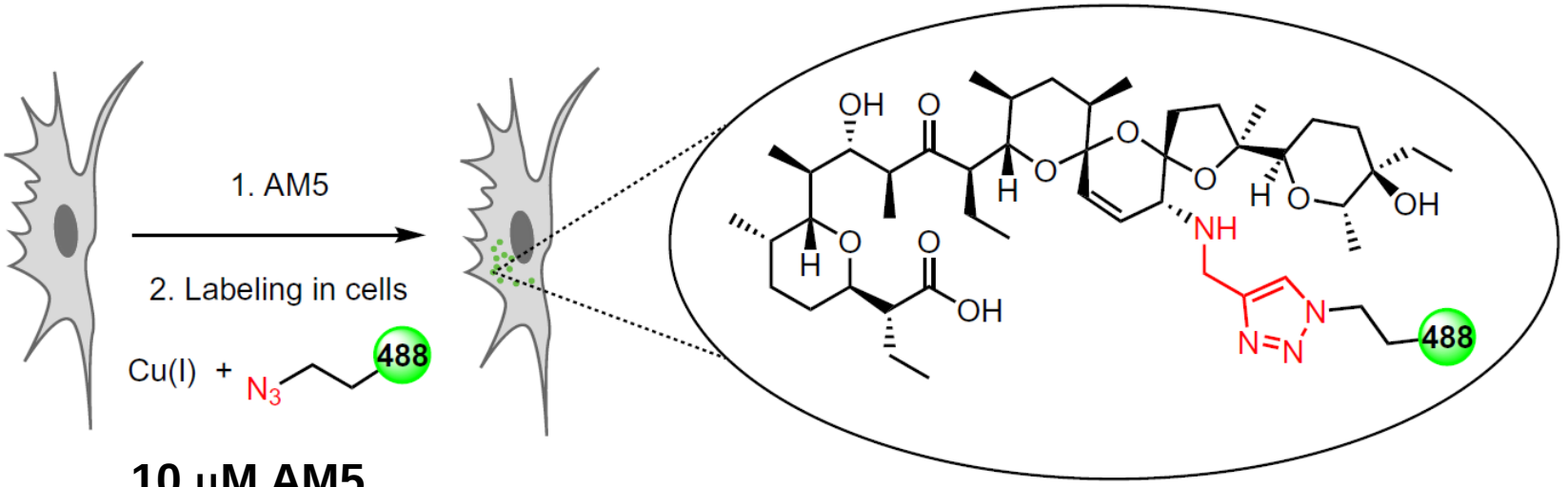




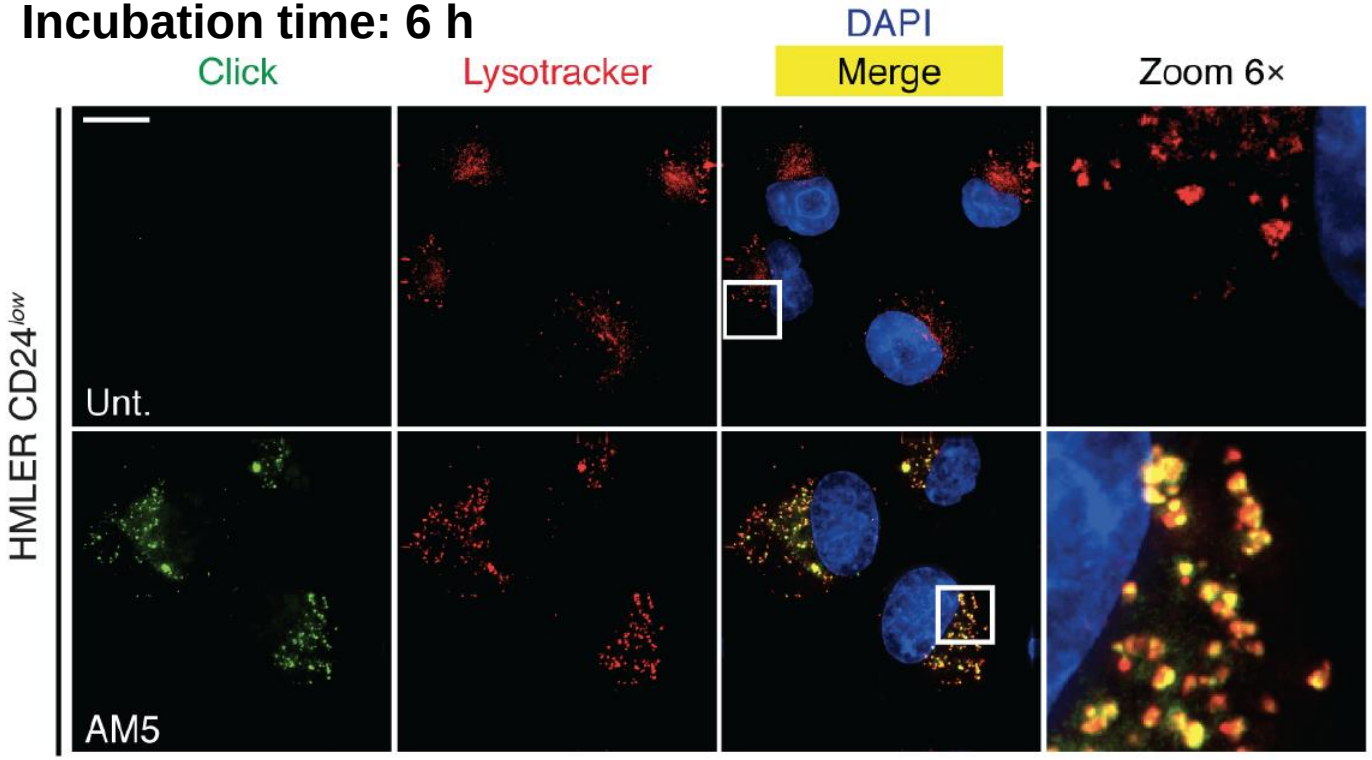
Ionophore activity.



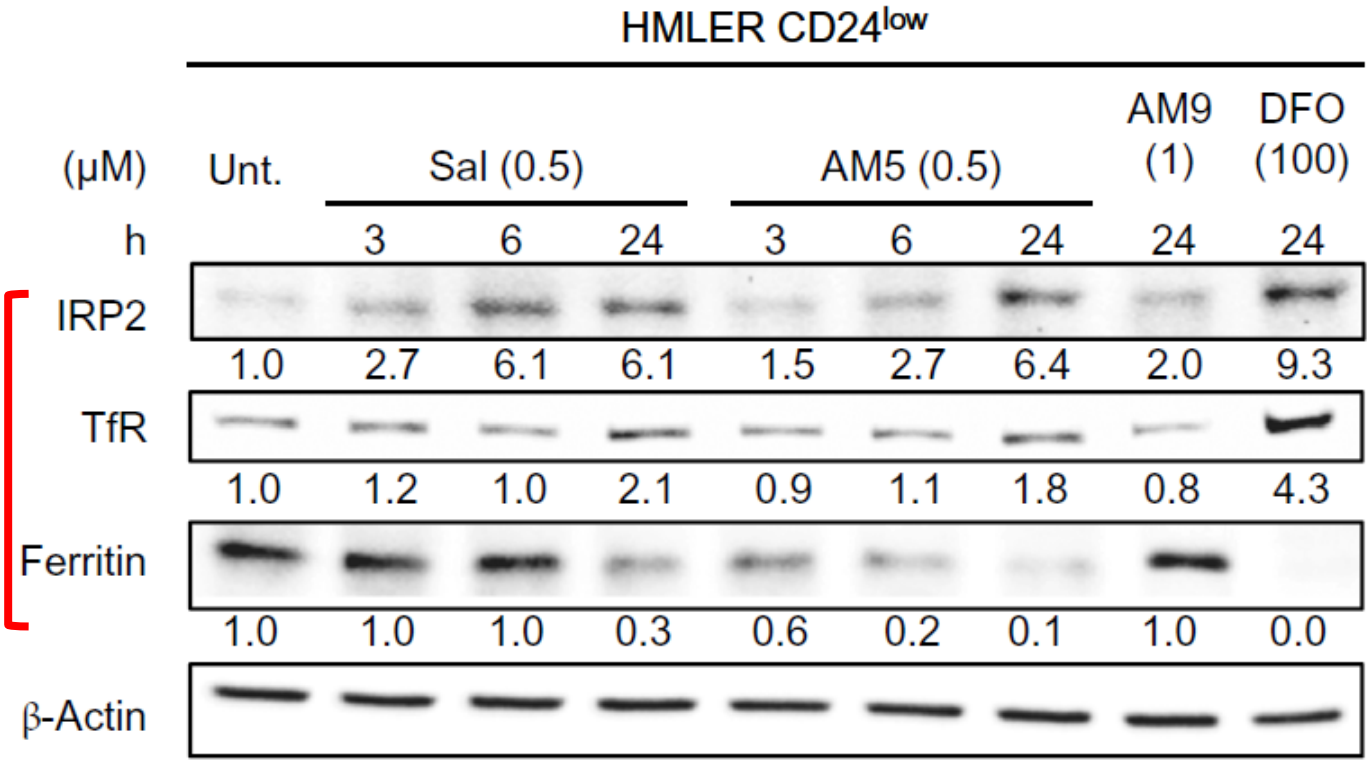
2-4. In Situ Huisgen Cycloaddition of AM5



10 μM AM5
Incubation time: 6 h



2-5. Immunoblotting

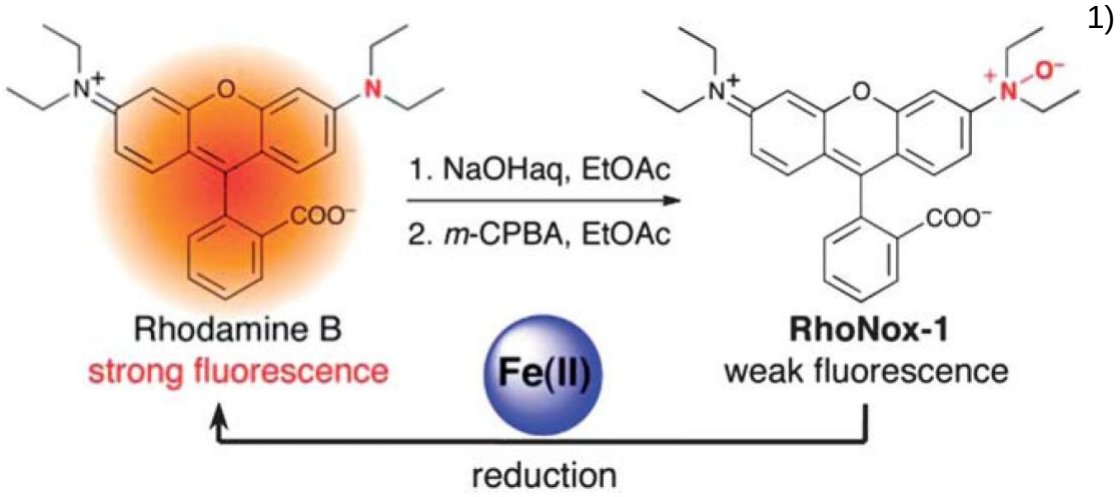


Iron homeostasis regulatory proteins

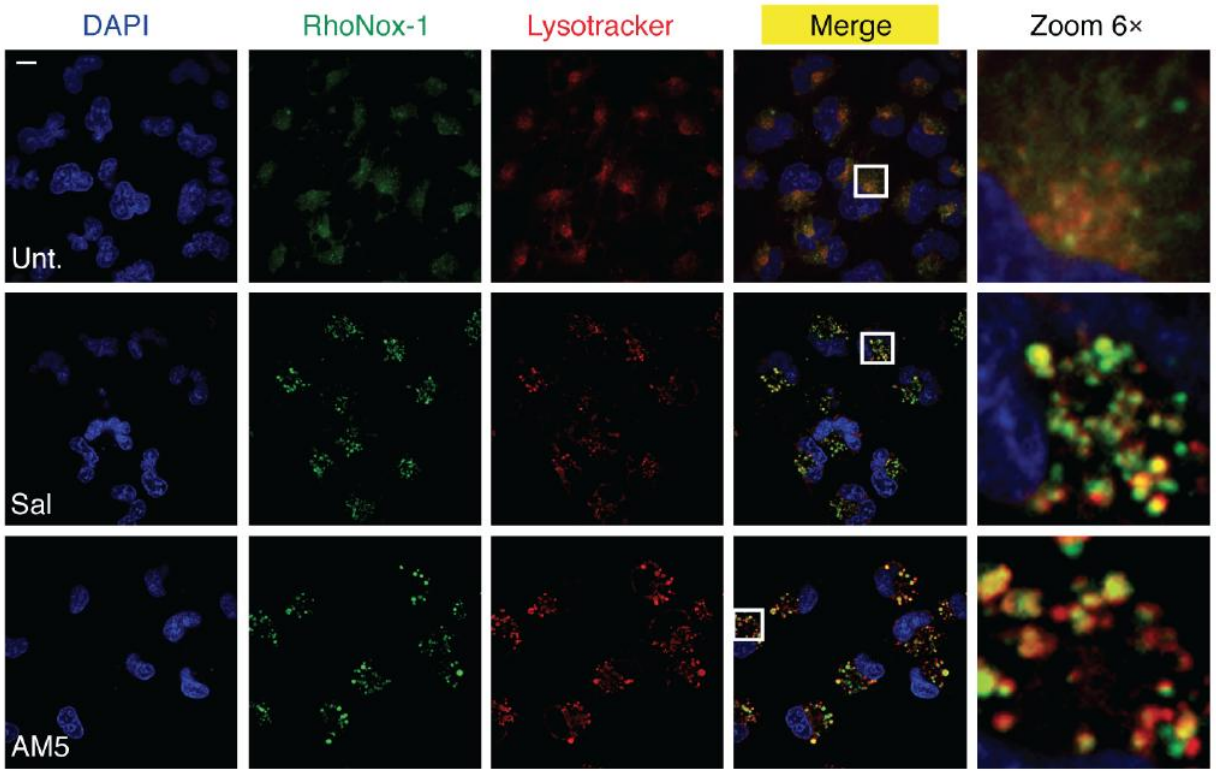
Cytoplasmic depletion of iron was indicated.

IRP2: iron-responsive element-binding protein 2
TfR: transferrin receptor
Ferritin: degraded and release Fe in lysosome
DFO: deferoxamine (iron-chelating reagents)

2-6. Fe (II) probe RhoNox-1



1)

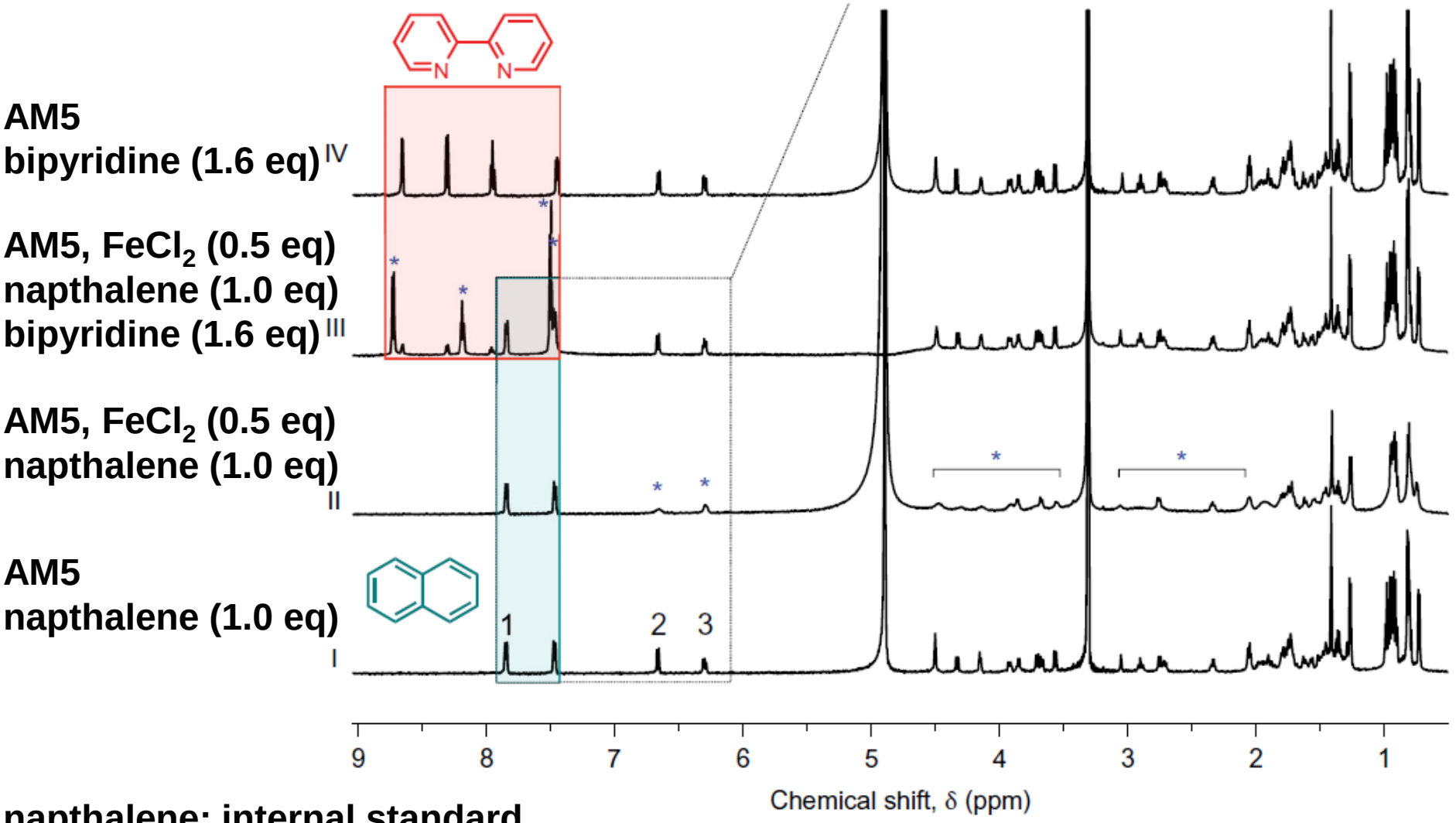


0.5 μM Sal or AM5
Incubation time: 48 h

Fe (II) accumulated in lysosome.

(1) Hirayama, T.; Okuda, K.; Nagasawa, H. *Chem. Sci.* **2013**, *4*, 1250.

2-7. NMR analysis

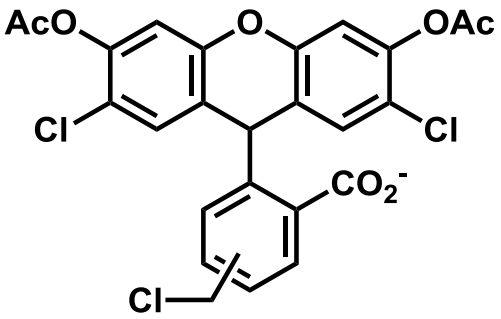


napthalene: internal standard
bipyridine: iron (II) chelator

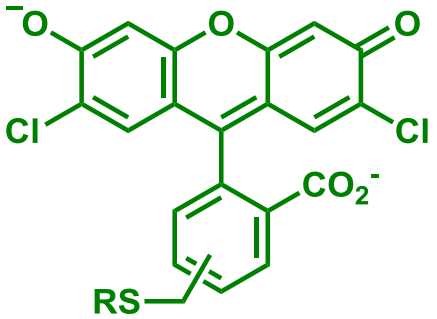
Paramagnetic relaxation enhancement indicate AM5-Fe(II) complex.

2-8. Lysosomal Reactive Oxygen Species (ROS)

CM-H2DCFDA (ROS probe)

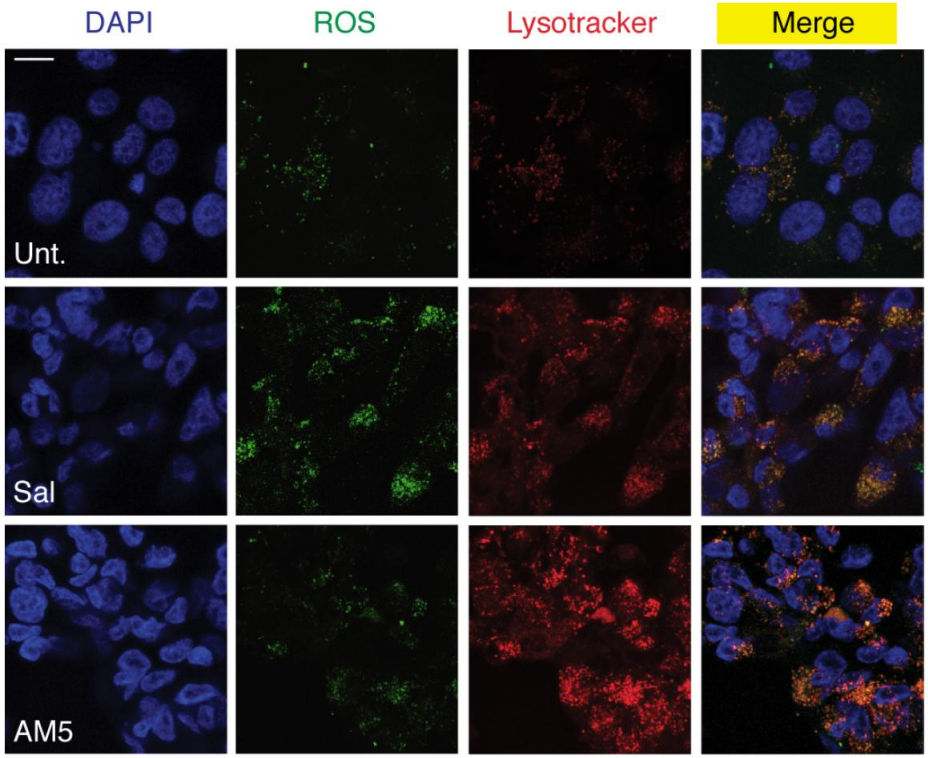


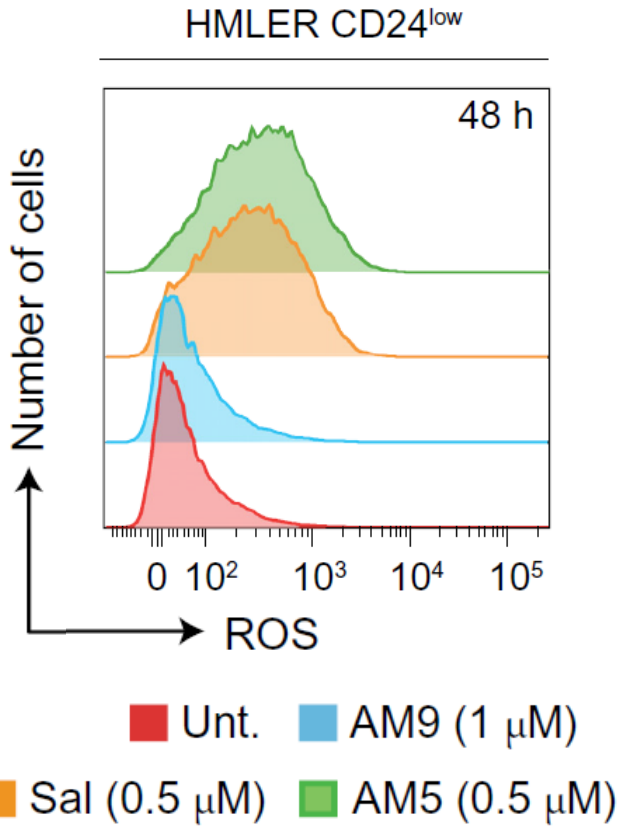
1) esterase
2) ROS



non-fluorescent

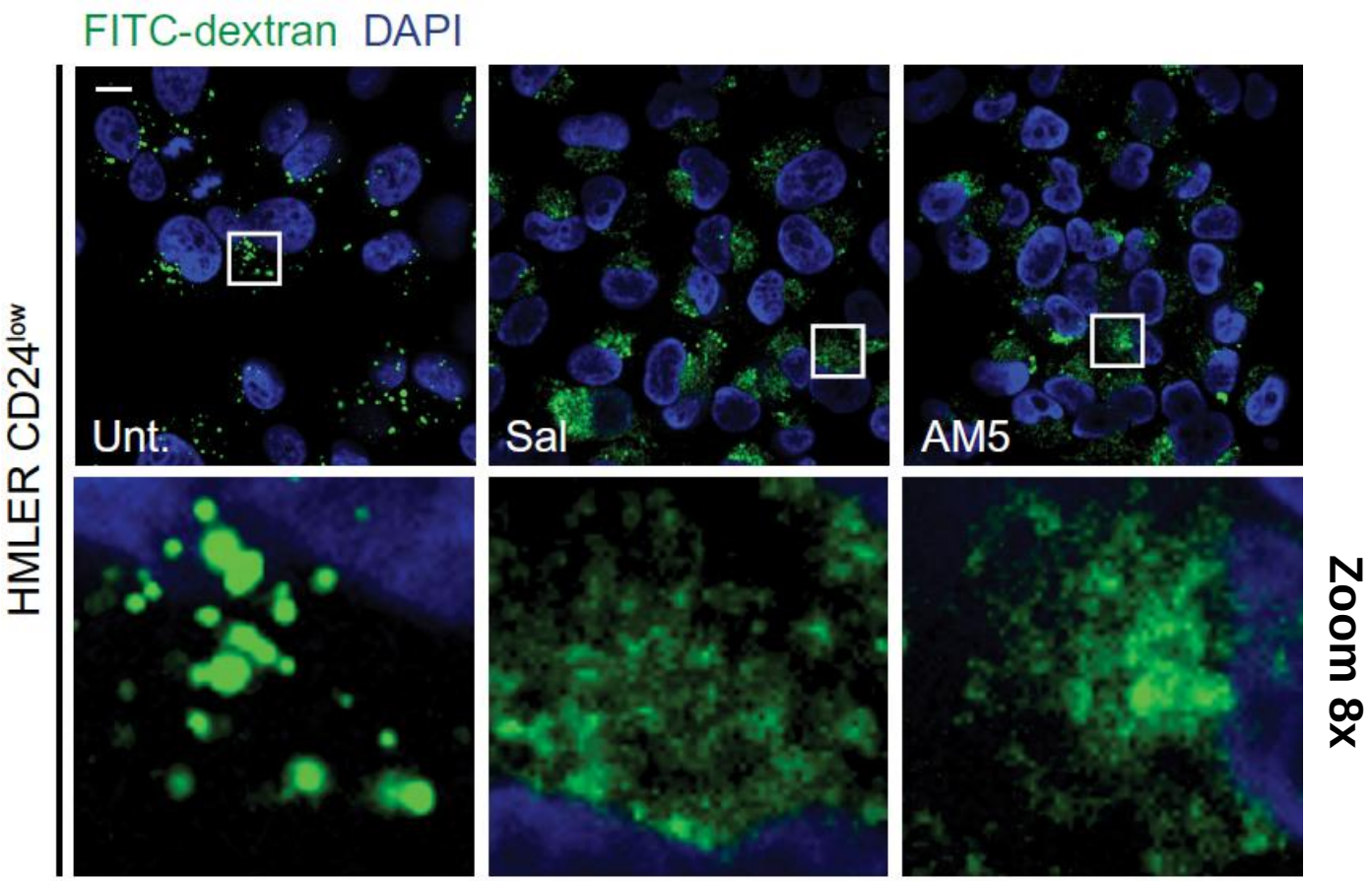
fluorescent





0.5 μM Sal or AM5
Incubation time: 48 h

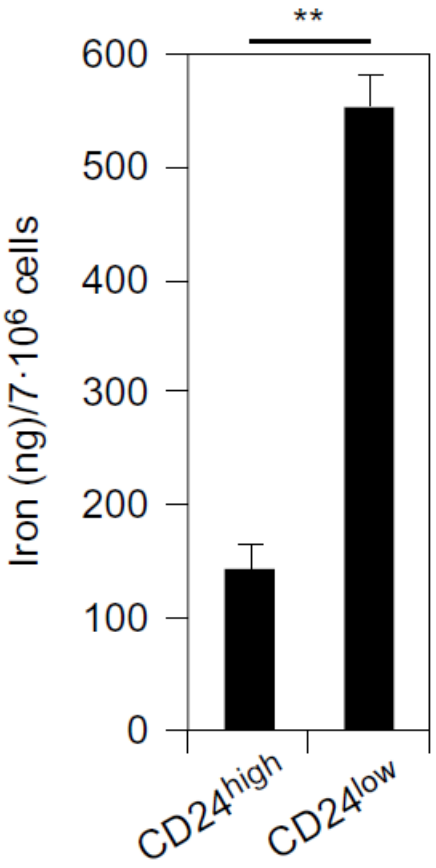
2-9. Lysosomal Membrane Permeabilization (LMP)

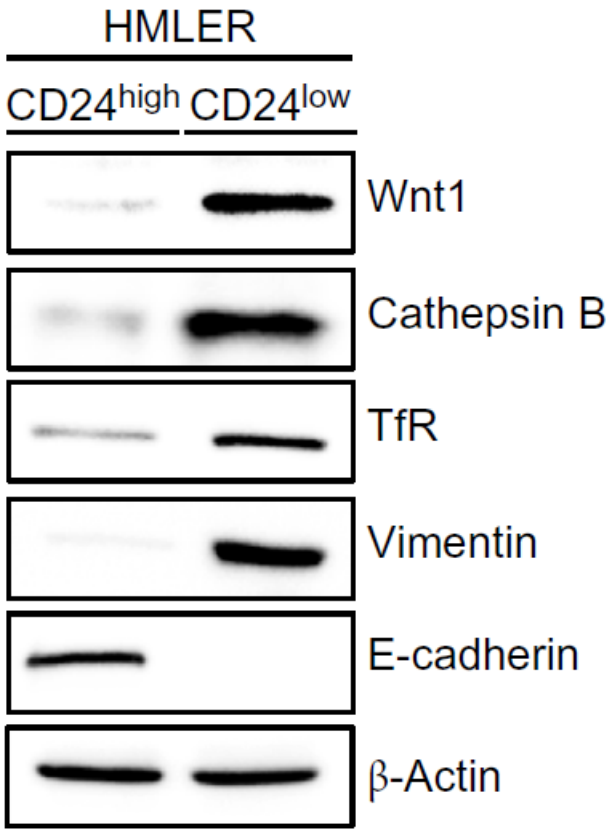


0.5 μM Sal or AM5
Incubation time: 48 h

Translocation of membrane-impermeable FITC-dextran suggest LMP.

2-10. CSC specific toxicity





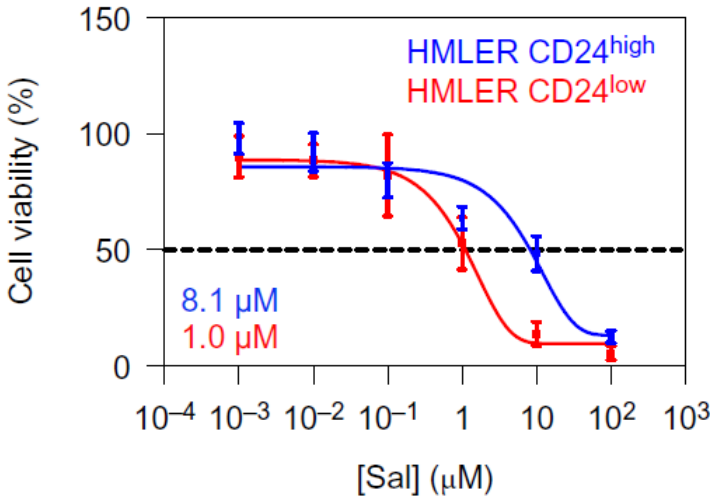
degraded Ferritin and release Fe in lysosome

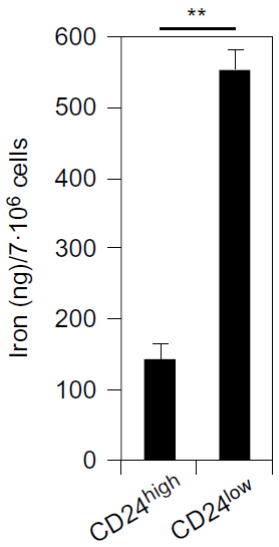
involved in iron-uptake

Mesencymal marker

Epithelial marker

2-11. Summary



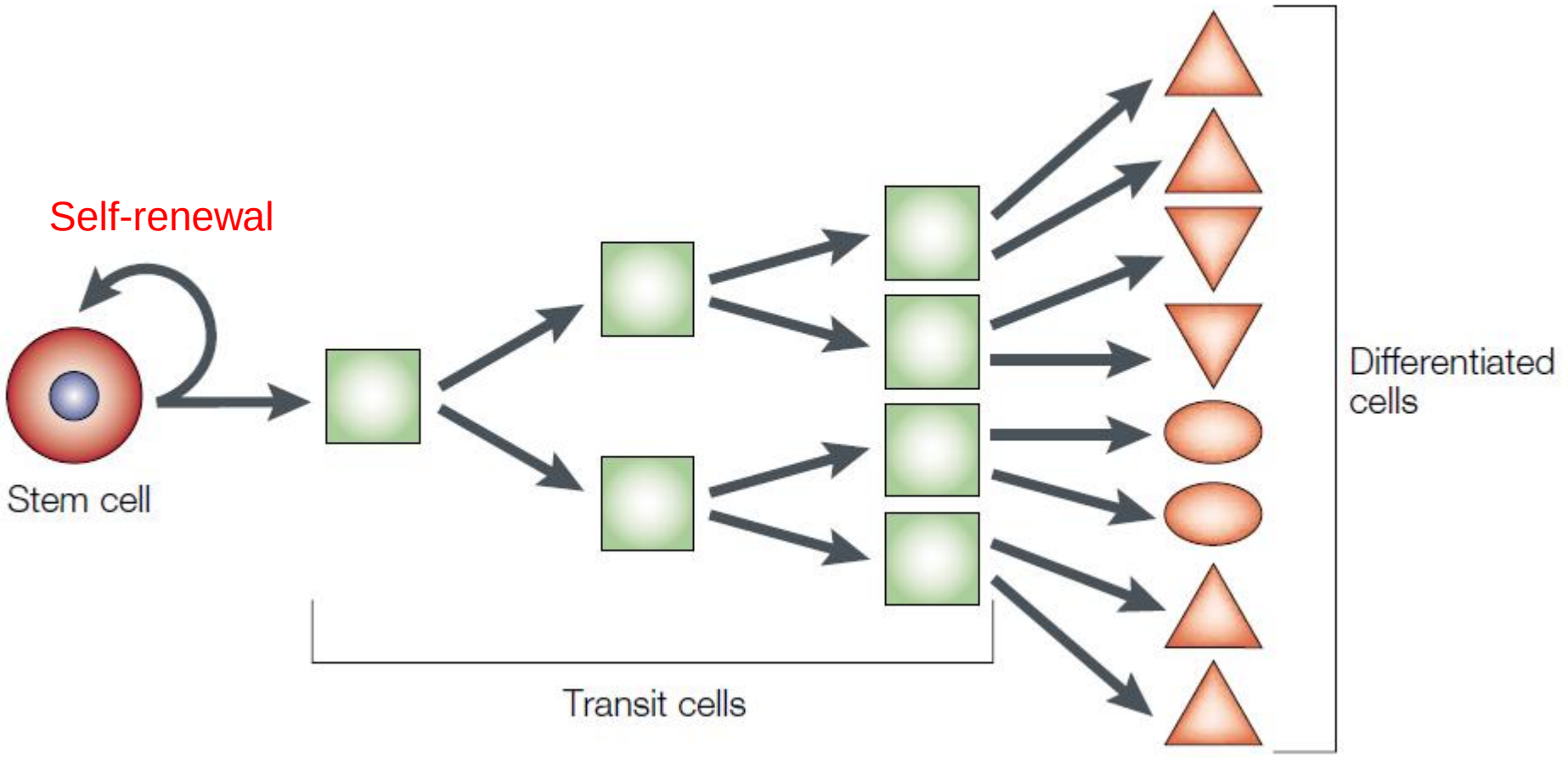


- **Salinomycin possessed specific toxicity against CSCs by disrupting iron homeostasis.**
- **Higher level of iron in CMC model cells suggest a function of iron in the maintenance of CMCs.**

Remaining unclear

- **Why Salinomycin was localized at lysosome?**
- **Structure and binding affinity of salinomycin-Fe(II) complex.**
- **Ionophore activity of salinomycin in lysosome.**

A1. Stem Cells

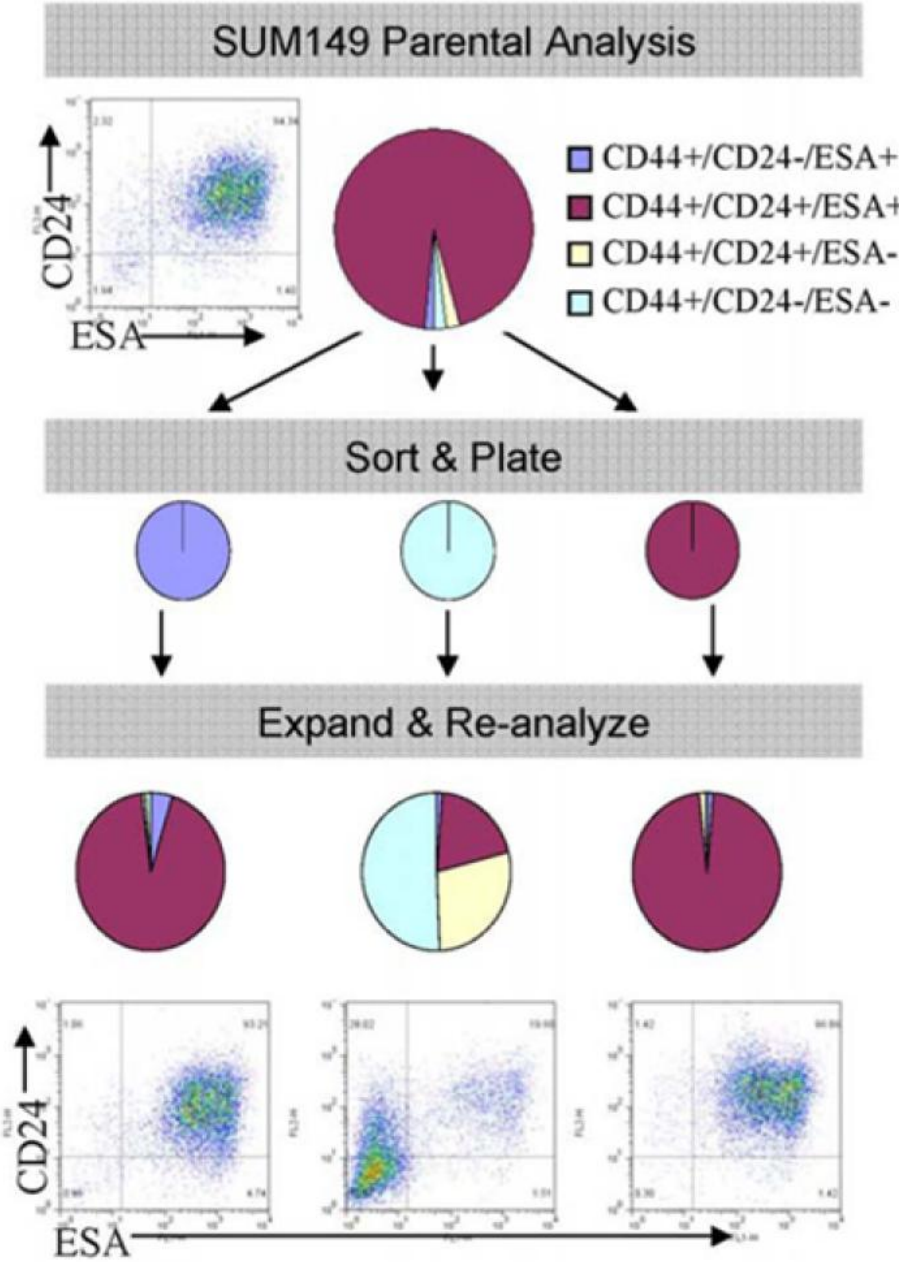


Stem Cells are defined as those cells that are undifferentiated and can divide to produce further stem cells, as well as cells that are destined to become differentiated cells. [1)]

(1) Tosh, D.; Slack, J. M. W. *Nature Reviews Molecular Cell Biology* **2002**, *3*, 187.

A2. CSCs in vitro culture

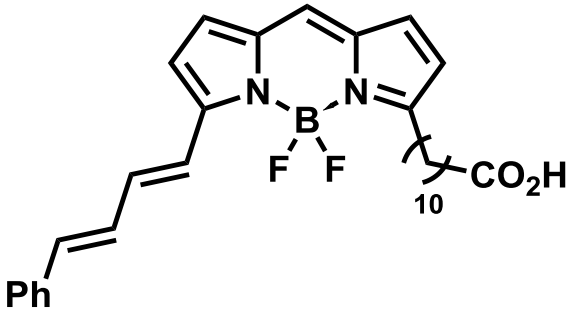
CD24, CD44: cell adhesion molecule
ESA: epithelial-specific antigen
SUM149: breast cancer cell lines developed from primary breast cancers



(1) Fillmore, C. M.; Kuperwasser, C. *Breast Cancer Research 10*, R25.

A3. Lipid ROS probe

BODIPY 581/591 C11 (Lipid ROS probe)



Em. ~590 nm —ROS→ **Em. ~510 nm**